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Mechanistic pharmacokinetic modelling of ephedrine, norephedrine and caffeine in healthy subjects

C. Csajka,[1] C. A. Haller,[2] N. L. Benowitz[1,2] & D. Verotta[1,3]

[1]*Department of Biopharmaceutical Sciences, University of California, San Francisco, CA,* [2]*Department of Medicine, Division of Clinical Pharmacology, University of California, San Francisco, CA,* [3]*Department of Biostatistics, University of California, San Francisco, CA, USA*

Correspondence
Davide Verotta, PhD, Department of Biopharmaceutical Sciences and Biostatistics, University of California, Box 0446, San Francisco CA 94143, USA.
Tel: +1 415 476 1556
Fax: +1 415 476 1508
E-mail: davide@ariel1.ucsf.edu

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Aim
The combination of ephedrine and caffeine has been used in herbal products for weight loss and athletic performance-enhancement, but the pharmacokinetic profiles of these compounds have not been well characterized. This study aimed to develop a mechanistic model describing ephedrine, norephedrine, and caffeine pharmacokinetics and their interactions in healthy subjects.

Methods
The pharmacokinetic model was developed based on the simultaneous modelling using plasma samples gathered from two clinical trials. The treatments consisted of single-doses of pharmaceutical caffeine and ephedrine, given alone or together, and an herbal formulation containing both caffeine and ephedrine. We used a mixed-effect statistical model and the program NONMEM to take account of intersubject variability.

Results
Three hundred and seventy-nine ephedrine, 352 norephedrine, 417 caffeine plasma concentrations and 40 ephedrine urine concentrations were obtained from 24 subjects. A one-compartment model with first-order absorption described the caffeine data. Caffeine clearance was 0.083 l min^{-1} (CV 38%) and decreased to 0.038 l min^{-1} in presence of oral contraceptive therapy, its volume of distribution was 38.6 l (CV 20%) and its absorption rate constant was 0.064 l min^{-1} (CV 50%). A four-compartment model described the pharmocokinetics of ephedrine and norephedrine. Ephedrine was eliminated mostly renally, with a clearance of 0.34 l min^{-1} (CV 11%), and a volume of distribution of 181 l (CV 19%). Nonlinearity in the conversion of ephedrine to norephedrine was observed. Different models showed that the simultaneous administration of caffeine, or the amount of caffeine in the absorption compartment, was associated with a slower rate of absorption of ephedrine. A 32% greater relative bioavailability of herbal compared with pharmaceutical ephedrine administration was observed.

Conclusions
We describe a mechanistic model for ephedrine, norephedrine and caffeine pharmacokinetics and their interactions. The relative bioavailability of ephedrine differed between the herbal supplement compared with the pharmaceutical formulation. Concomitant ingestion of caffeine slowed the absorption rate of ephedrine, which is mainly related to the amount of the former in the absorption compartment. A saturable process appears to be involved in the metabolism of ephedrine to norephedrine.

Introduction

Ephedrine and caffeine are the primary active constituents of herbal dietary supplements that contain Ma Huang (*Ephedra sinica*) and guarana (*Paullinia cupana*). The simultaneous use of these thermogenic drugs has been promoted for weight loss and for enhancement of athletic performance, although their use has been associated with a significant number of severe side-effects [1–9]. Several noncompartmental studies investigating the pharmacokinetics of ephedrine have been reported in the literature [10–14] as well as one study using a simple model and a novel population approach [15]. A few studies have investigated the combination of ephedrine with caffeine for its efficacy as weight loss and ergogenic agents [16–18]. Recently, we showed that pharmacokinetic and pharmacodynamic interactions exist between caffeine and ephedrine [13, 14]. However, no study has characterized the pharmacokinetics of ephedrine and of its major metabolite norephedrine, nor modelled the interaction between caffeine and ephedrine. Accordingly, the objectives of this study were thus to use data gathered from our previous clinical studies [13, 14] (1) to develop a more mechanistic model for ephedrine, norephedrine and caffeine pharmacokinetics; (2) to investigate whether there are differences in pharmacokinetic parameters after ingestion of the pharmaceutical compared with the herbal formulations of caffeine and ephedrine, and (3) to investigate the mechanism and the magnitude of the effect of caffeine on the absorption of ephedrine.

Materials and methods

Study population

Population pharmacokinetics of ephedrine, norephedrine and caffeine were characterized using plasma concentrations gathered from two clinical studies that have been described previously [13, 14]. In brief, the first study involved eight subjects, who received a single oral dose (two capsules) of a commercial dietary supplement (Metabolift®) labelled to contain 200 mg caffeine and 20 mg ephedrine alkaloids. By analysis, this dose consisted of 17.3 mg ephedrine, 0.2 mg norephedrine, 5.3 mg pseudoephedrine, 0.42 mg norepseudoephedrine and 175 mg caffeine. In the second study, single oral doses of either 25 mg ephedrine sulphate (West-ward Pharmaceutical Corp., Eatontown, NJ, USA) or 200 mg caffeine sulphate were administered alone and together to 16 subjects. Blood samples were collected immediately prior and at 30, 1, 1.5, 2, 4, 6, 8, 11, 12, 14 and 18 h after drug intake. Urine was collected from 0 to 14 h and from 0 to 24 h during the first and the second studies, respectively. Quantitative measurements of ephedrine, its active metabolite norephedrine, and caffeine were performed on all blood samples and the total (14 h and 24 h) urine collections for ephedrine. In total, 379 ephedrine, 352 norephedrine and 417 caffeine plasma samples and 40 urine samples were assayed. Urine pH was measured in each urine collection void over the study period. Two subjects enrolled in the first study and four in the second study (25%) were taking an oral contraceptive pill, and thus we were able to investigate the effects of this commonly used medication on the pharmacokinetics of caffeine. All subjects were advised of the risks of the study and gave written informed consent before enrolment. The committee on Human Research at the University of California, San Francisco, approved these studies. The demographic characteristics of the patients are presented in Table 1.

Drug analysis

Determination of the doses of ephedrine and caffeine present in the herbal formulations as well as plasma concentration measurements of ephedrine, norephedrine, and caffeine was by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method that has been described previously [13]. The limit of determination was 0.5 ng ml^{-1} for ephedrine and norephedrine, and 25 ng ml^{-1} for caffeine. Precision and accuracy were evaluated by replicate analysis of spiked plasma samples at three concentrations that spanned the concentration ranges for the analytes in clinical study samples. Within-run precision (coefficient of variation, $n = 6$) ranged from 0.16 to 2.5%. Accuracy (percentage of expected values; $n = 6$) ranged from 97 to 105%.

Caffeine pharmacokinetics

Caffeine pharmacokinetics were characterized using a simple one-compartment model with first order absorption. The model is expressed using differential equations as follows:

$$\frac{dA_1}{dt} = -ka_C A_1;\ A_{10} = dose_C F_C \quad (1)$$

$$\frac{dA_2}{dt} = ka_C A_1 - \frac{CL_C}{V_C} A_2;\ A_{20} = C_0 V_C \quad (2)$$

$$C = \frac{A_2}{V_C} \quad (3)$$

where A_1 and A_2 denote amounts in the absorption and plasma compartments, respectively, ka_C the first order absorption rate constant, CL_C the plasma clearance, V_C the volume of distribution, F_C is the absolute bioavailability of caffeine, A_{10} and A_{20} the initial conditions in

the absorption and plasma compartments, $dose_C$ the dose of caffeine, C_0 the baseline concentration of caffeine at time zero, and C the observed concentration of caffeine. Since no intravenous drug concentration data were available, the absolute bioavailability could not be estimated and CL_C and V_C represent apparent values (CL_C/F_C, V_C/F_C).

The influence of oral contraceptive treatment (*OC*) on CL_C was modelled as follows:

$$CL_C(1-\theta_{OC}^{CL}OC) \quad (4)$$

where *OC* is an indicator variable that takes the value of 1 during oral contraceptive therapy and 0 in its absence, and θ_{OC}^{CL} $(0 \le \theta_{OC}^{CL} \le 1)$ is the fractional decrease in CL_C in presence during oral contraceptive therapy.

Ephedrine and norephedrine pharmacokinetics

Ephedrine and norephedrine pharmacokinetics were assessed using a four-compartment model. The conversion of ephedrine to norephedrine was modelled according to the Michaelis-Menten equation to account for saturable metabolism. A schematic representation of the compartmental model for ephedrine, norephedrine and caffeine pharmacokinetics is shown in Figure 1. The rate of change of ephedrine (E) and norephedrine (NE) concentrations was modelled as follows:

$$\frac{dA_3}{dt} = -ka_E A_3; A_{30} = dose_E F_E \quad (5)$$

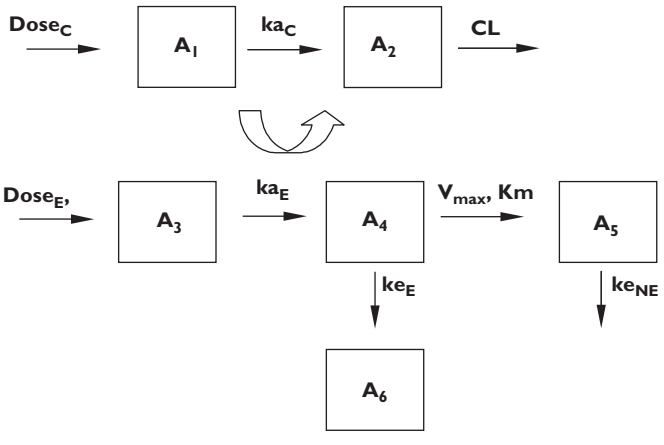

Figure 1

Schematic representation of the compartmental model for caffeine, ephedrine and norephedrine pharmacokinetics, where A_x represents the amounts in the *x* compartment. A_1: caffeine absorption; A_2: plasma caffeine; A_3: ephedrine absorption; A_4: plasma ephedrine; A_5: plasma norephedrine; A_6: urine ephedrine. The curved arrow indicates the influence of caffeine on ephedrine absorption

$$\frac{dA_4}{dt} = ka_E A_3 - \frac{V_{max}A_4}{A_4 + Km} - \frac{CL_{RE}}{V_E}A_4; A_{40} = 0 \quad (6)$$

$$\frac{dA_5}{dt} = \frac{V_{max}A_4}{Km + A_4} - ke_{NE}A_5; A_{50} = 0 \quad (7)$$

$$\frac{dA_6}{dt} = \frac{CL_{RE}}{V_E}A_4 \quad (8)$$

$$E = \frac{A_4}{V_E}; E_u = A_6; NE = \frac{A_5}{V_{NE}}$$

where A_3 is the amount of ephedrine in the absorption compartment, A_4 and A_5 are the amounts of ephedrine and norephedrine, respectively, in the plasma compartment, A_6 is the amount in the urine compartment, $dose_E$ is the dose of ephedrine, ka_E is the first order absorption rate constant, CL_{RE} is the renal clearance, F_E is a scaling factor determining the fractional dose recovered in the urine, V_{max} and Km are the maximal rate of metabolism and the Michaelis-Menten constant, respectively, and E and NE are the observed ephedrine and norephedrine plasma concentrations. Because norephedrine is not administered, its volume of distribution (V_{NE}) cannot be determined and thus V_{max} is a compound parameter that incorporates V_{NE} [19].

To account for any difference in the pharmacokinetic profiles of ephedrine and caffeine after administration of the herbal compared with the pharmaceutical formulations, we estimated apparent bioavailabilities for both formulations (F_E,pharm) and (F_E,herbal). The relationship between individual measurements of urine pH (collected at different time during the study) and individual (empirical Bayes) estimates of renal clearance was assessed by linear regression using the equation $CL_R = a + b\ pH$ (S-PLUS Statistical Sciences, Version 4.0 Release 2 1997, http://www.statsci.org/splus.html). Statistical significance was defined as $P < 0.05$.

Interaction of caffeine and ephedrine

The influence of caffeine on the rate of absorption of ephedrine was characterized using a class of models related to so-called indirect action models, as described by Jusko [20]. We tested different models of the general form:

$$\frac{dA_3}{dt} = -ka_E(1 - f(C))A_3 \quad (9)$$

where f(C) indicates a generic function of caffeine. The first model we considered simply allows a different value for the absorption rate in presence of caffeine, and it was coded as follows:

$$f(C) = \delta I(dose_C) \quad (10a)$$

where $I(dose_C)$ is an indicator variable that equals 1 when caffeine is administered and zero when it is not, and $\delta = \frac{\exp^q}{1+\exp^q}$, $0 \le \delta \le 1$, represents the fractional reduction of ka_E due to the presence of caffeine. Different semiempirical models relate absorption rate to the amount of caffeine in the absorption compartment (A_1) or plasma compartment (A_2) or to total amount ($A_1 + A_2$) as follows:

$$f(C) = \delta \frac{A_1}{A_1 + ka_{E,50}} \quad (10b)$$

$$f(C) = \delta \frac{A_2}{A_2 + ka_{E,50}} \quad (10c)$$

$$f(C) = \delta \frac{A_1 + A_2}{A_1 + A_2 + ka_{E,50}} \quad (10d)$$

$$f(C) = \delta(1 - \exp^{-A_1 0.693/ka_{E,50}}) \quad (10e)$$

where $ka_{E,50}$ represents the amount (of A_1, A_2, $A_1 + A_2$ or A_1 in model 10b, 10c, 10d or 10e, respectively) resulting in a 50% ($\delta/2$) fractional decrease in the absorption rate, and for all models $0 \le f(C) \le 1$, $f(0) = 0, \lim_{C\to\infty} \le f(C) = 1$.

Variance models

To take into account intersubject variability we use a mixed effect model, implemented using the computer program NONMEM [21]. The pharmacokinetics parameters for the j^{th} individual were modelled according to the following equations:

$$CL_j = CL e^{\eta_{1j}} \qquad V_{Ej} = V_E e^{\eta_{4j}}$$
$$V_{Cj} = V_C e^{\eta_{2j}} \qquad Km_j = Km e^{\eta_{5j}}$$
$$ka_{Cj} = ka_C e^{\eta_{3j}} \qquad ke_{NEj} = ke_{NE} e^{\eta_{6j}}$$

where η_{1j}.,η_{6j} are independent normally distributed effects with mean zero and variance $\Omega_1, \ldots, \Omega_6$.

The individual elimination and absorption rates of ephedrine were modelled according to:

$$\frac{CL_{RE}}{V_{E_j}}(t) = \left(\frac{CL_R}{V_E} + \frac{V_{max}}{A_4(t) + Km}\right) e^{\eta_{7j}} \quad (11)$$

$$ka_{E_j}(t) = ka_E[1 - f(C(t))] \times \left[I(dose_C) e^{\eta_{8j}} + (1 - I(dose_C)) e^{\eta_{9j}}\right] \quad (12)$$

where η_{7j}, η_{8j}, η_{9j} are assumed to be normally distributed with mean zero and variance Ω_7 and $\Omega_8 = \Omega_9$, and t indicates that theses rates are ultimately a function of time. In equation 11, we assume that individuals differ for an overall scaling factor ($e^{\eta_{7j}}$) associated with total clearance. In equation 12, we allow for a similar individual variation of overall absorption rate that is defined by the factor $e^{\eta_{8j}}$ and $e^{\eta_{9j}}$ in the presence or absence of caffeine, respectively. To simplify the model due to the small number of subjects involved in the study we assume that the variance of η_{8j} and η_{9j} is the same.

Proportional error models following a normal distribution were assumed for the description of the residual intrasubject residual variabilities for caffeine, norephedrine and ephedrine, for which a separate error was introduced to differentiate plasma and urine concentrations. The i^{th} concentration measurements from the j^{th} individual were modelled as follows:

$$C_{pji}(1 + \varepsilon_{1ji})$$
$$C_{uji}(1 + \varepsilon_{2ji})$$
$$E_{ji}(1 + \varepsilon_{3ji})$$
$$NE_{ji}(1 + \varepsilon_{4ji})$$

where C_{pji}, $E_{ji,}$ $NE_{ji,}$ are the corresponding predicted i^{th} plasma concentration and C_{uji} the predicted urine concentration for the j^{th} individual, and $\varepsilon_{1ij,}$ ε_{2ij}, ε_{3ij}, ε_{4ij} are independent normally distributed residual error terms with a mean of zero and a variance of Σ_1, Σ_2, Σ_3, Σ_4.

Parameter estimation and model selection

The modelling of the plasma concentrations of ephedrine, norephedrine and caffeine were assessed in two separate analyses by use of the NONMEM software [21]. First, we modelled caffeine concentrations, and second, conditional on the predictions for caffeine, we modelled ephedrine and norephedrine concentrations (Figure 1). The amounts of caffeine A_1 and A_2 over time were obtained from its final posthoc individual pharmacokinetic parameters (CL_C, V_C, ka_C and lag time).

The models were fitted to the data using the first order conditional method with interaction (FOCE INTERACTION) and the subroutine ADVAN6. NONMEM performs linearized maximum likelihood estimation by use of an objective function (OF). To determine whether there was a statistically significant difference

between the goodness of fit between the two models, we used the Akaike model selection criteria [22] which require a decrease of two points in the objective function (minus twice the logarithm of the likelihood of the model) to accept a model with one additional parameter, as well as a comparison of diagnostic plots (observed concentrations compared with predictions and observations/predictions compared with time). Typical values (population means) with their corresponding standard errors (SE), and the intersubject and intrasubject variabilities were expressed as coefficients of variation (CV percentage). The figures were generated with S-PLUS (Statistical Sciences, Version 4.0 Release 21997).

Results

Caffeine pharmacokinetics

Plasma caffeine concentrations ranged from 0 to 8470 μg l^{-1}. Residual caffeine concentrations (range: 14.2–3820 μg l^{-1}) were detected prior to dosing in 22 subjects and were integrated into the model as baseline concentrations (see equation 2). A one-compartment model with first-order absorption described the caffeine data appropriately and no further improvement was detected when using a two-compartment model (the decrease in the objective function, ΔOBJ, for the two-compartment model was 0.1). Introducing a pure absorption lag time (ΔOBJ = −17) or a different lag time for herbal caffeine compared with caffeine citrate (ΔOBJ = −36) improved the fit, indicating a delay in absorption of 22.2 min after intake of former as opposed to no delay after intake of the latter. Assignment of concurrent therapy with oral contraceptives as a dichotomous covariate on CL_C resulted in a significant 46% decrease in CL_C (ΔOBJ = −13) in patients taking oral contraceptives, as well as a decrease of 15% in the interindividual variability in CL_C. The population mean estimate of CL_C was 0.083 l min^{-1} with a decrease to 0.038 l min^{-1} (CV 38%) during oral contraceptive therapy, V_C was 38.6 l (CV 20%) and ka was 0.064 1 min^{-1} (CV 50%). The corresponding elimination half-life was 5.3 h which increased to 11.8 h in oral contraceptive users. Concentrations *vs*. time profiles after administration of herbal caffeine and caffeine citrate, along with the population predictions, are shown in Figure 2.

Ephedrine and norephedrine pharmacokinetics

Plasma ephedrine and norephedrine concentrationsranged from 1.59 to 101.40 mg l^{-1}, and from 0.51 to 8.18 mg l^{-1}, respectively. The amount of ephedrine excreted in the urine ranged from 7.35 to 22.2 mg. A two-compartment model with first-order absorption and first order elimination described the ephedrine data appropriately (ΔOBJ = −1 for 1 compared with two compartments distribution). The assignment of absorption lag-time visually improved the fits (ΔOBJ = −78). Modelling the formation of norephedrine from ephedrine using a first order process resulted in a poor fit of the norephedrine plasma data (Figure 3, dotted line). The rate of conversion of ephedrine to norephedrine was thus modelled using a Michaelis-Menten equation (see equation 6) and resulted in a significant drop in the objective function (ΔOBJ = −124) and an improved fit to the data (Figure 3, solid line).

Population mean estimates for the pharmacokinetic parameters were CL_{RE} 0.34 l min^{-1} (CV = 11%), V_E/F 181 l (19%), lag-time 16.7 min (22%), V_{max}/V_{NE} 1.96^{-4} μg min^{-1} l^{-1} (11%), Km 2.77 μg (45%) and ke_{NE} 0.037 1 min^{-1} (45%). From these population parameters, the half-life of ephedrine was estimated to be 6.1 h and its mean absorption lag-time without concomitant caffeine administration was 44 min.

We observed a linear association between individual measurements of urine pH (collected at the time of urine voiding) and individual (empirical Bayes) estimates for renal clearance, described by the equation $CL_R = a + b\ pH$. The coefficient *a* was to be 0.4723 (0.045)(l min^{-1}) and *b* −0.0172 (0.0069)(l min^{-1} U^{-1} pH), with an associated *P*-value of 0.013.

Influence of formulation

A separate analysis of the data from the first and second studies revealed a significant difference in both CL_R and V_E. Although the $t_{1/2}$ was similar, both CL_R and V_E decreased from 37.7 l h^{-1} to 22.7 l h^{-1} and from 320 l to 217 l when comparing the herbal and pharmaceutical ephedrine. Simultaneous modelling of the data from both studies resulted in a marked under prediction of the herbal ephedrine concentrations. The introduction of different bioavailabilities for the two formulations of ephedrine resulted in good predictions of the concentrations (ΔOBJ = −26). The apparent bioavailabilities of F_E,pharm and F_E,herbal were 0.59 and 0.78, respectively, indicating a 32% increase after intake of the herbal formulation of ephedrine compared with the pharmaceutical one. No significant intersubject variability was detected for these parameters and the variance was fixed to zero. The upper panel of Figure 4 shows the predictions of ephedrine concentrations after intake of the herbal formulation (solid line) and, for comparison, the dotted line shows the predictions when F_E,herbal is fixed to 1. No statis-

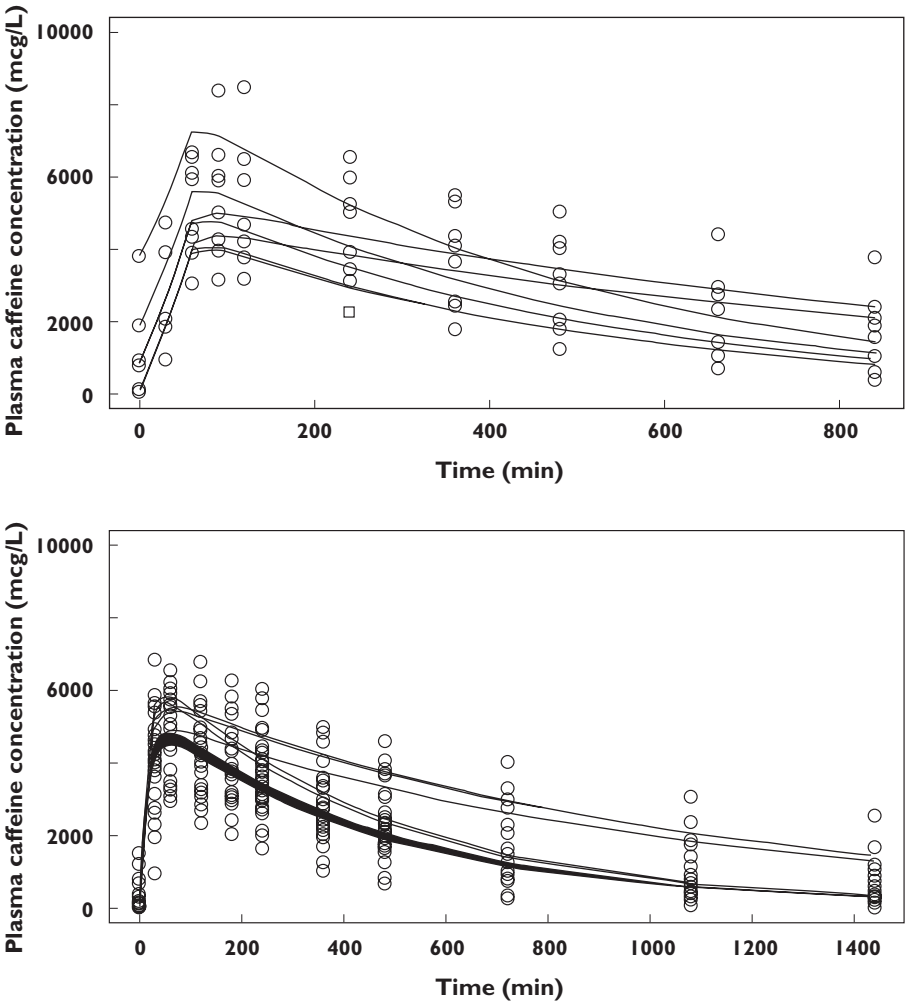

Figure 2
Caffeine plasma concentrations (open circles) and population prediction in subjects taking (solid line) and not taking (dashed line) oral contraceptives. Upper panel: herbal administration of caffeine in eight subjects. Lower panel: pharmaceutical administration of caffeine in 16 subjects. The different population prediction lines are the consequences of different baseline caffeine concentrations prior to dosing

tically significant discrepancies in the pharmacokinetic profile for norephedrine with regard to the different formulations were observed. A small influence of formulation on caffeine pharmacokinetics was noted, suggesting an increase in bioavailability of 19% after intake of herbal caffeine. However, owing to the small corresponding decrease in the objective function (ΔOBJ = −3.9) and no apparent difference in pharmacokinetic profiles for caffeine in the plots, this effect was not retained in the final model.

Interaction between ephedrine and caffeine

A statistically significant influence of caffeine on the absorption of ephedrine was observed. Models that introduced an effect of caffeine on the rate of ephedrine absorption rate (equation 10a–10e) resulted in a significant improvement in the fit to the data. For the simplest model (equation 10a), the absorption rate constant ka_E was 0.033 1 min^{-1} without caffeine and 0.020 1 min^{-1} (40% reduction) in the presence of caffeine (ΔOBJ = −124). Modelling ephedrine absorption as a function of caffeine in its absorption compartment using an indirect action model (either model 10b or 10e) resulted in an even better characterization of the data (ΔOBJ = −183). This model was chosen as the final model since no further improvement was observed when relating ephedrine absorption to the amount of caffeine in plasma (model 10c) or using the total amounts of caffeine (model 10d). The mean estimate for ka_E was 0.036 1 min^{-1}, with an intersubject variability of 63% (see equation 12). An asymptotic decrease of 99% in ka_E was estimated, due to caffeine present in the absorption compartment. The amount of caffeine at half of maximal decrease ($ka_{E,50}$) was 31.4 μg. The mean absorption profile of ephedrine with and without concomitant caffeine for the models corresponding to equation 4 and equation 10b is presented in Figure 4.

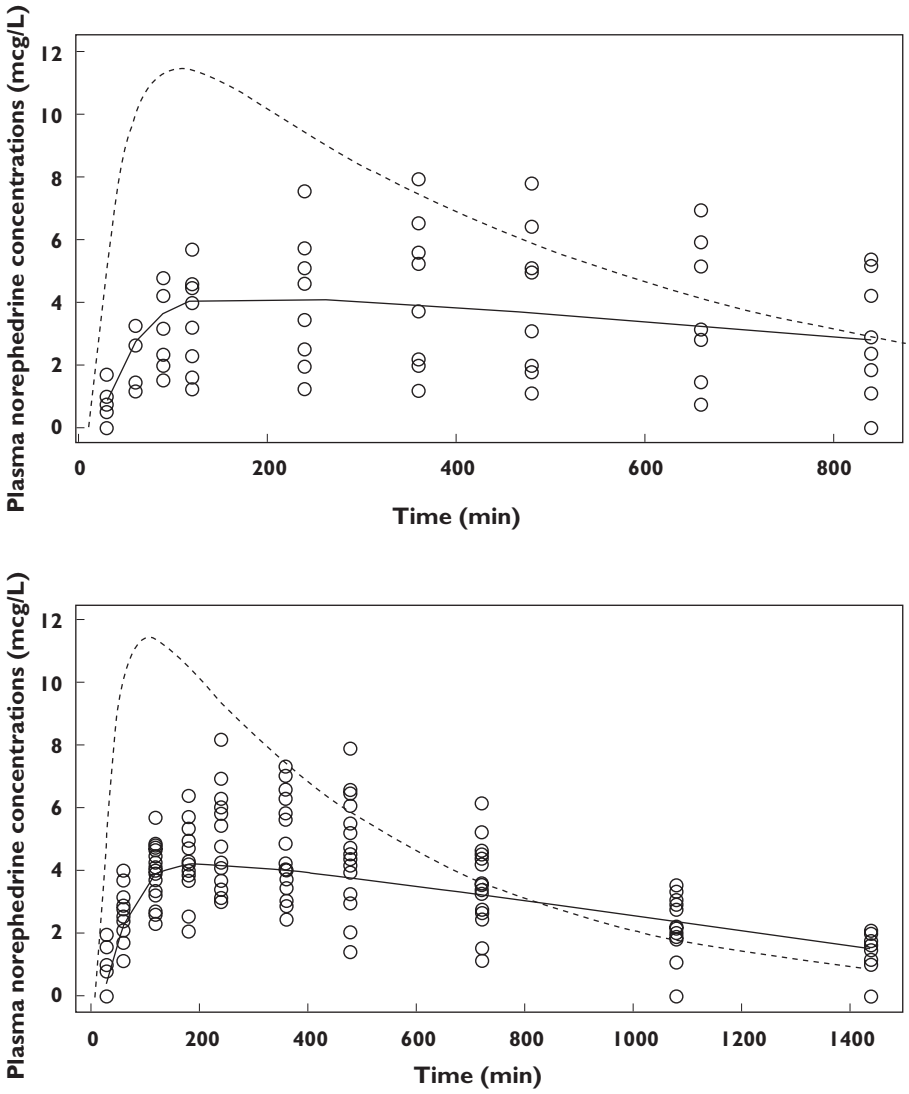

Figure 3
Plasma concentrations (open circles) and population prediction for norephedrine Upper panel: plasma concentrations for the eight subjects receiving herbal ephedrine. Lower panel: plasma concentrations for the 16 subjects receiving pharmaceutical ephedrine. Solid lines: predictions obtained by the final model assuming a nonlinear rate of conversion of ephedrine to norephedrine. Dotted lines: predictions obtained by the model assuming a constant rate of conversion from ephedrine to norephedrine

Table 1
Demographic characteristics of the 8 subjects enrolled in study 1 and the 16 subjects enrolled in study 2

	Study 1 (n = 8)	Study 2 (n = 16)
Age (years)	25–38	22–39
Body weight (kg)	52–88.9	58.6–91.5
Height (cm)	–	144–188
Sex	5 females/3 men	9 females/7 men
Race	–	10 Caucasian 1 African American 3 Asian/Pacific Islander 2 Latino

No statistically significant effect of ephedrine on caffeine absorption was observed.

The summary of the model selection steps characterizi ephedrine, norephedrine and caffeine pharmacokinetics is presented in Table 2.

The estimates of the population pharmacokinetic parameters for ephedrine, norephedrine and caffeine as well as those of the intersubject and intrasubject variabilities are presented in Table 3.

Discussion

Our study presents a mechanistic model for ephedrine, norephedrine and caffeine pharmacokinetics and for the interactions between these drugs. For caffeine, the $t_{1/2}$ of 5.4 h and the oral clearance of 5.0 l h^{-1} in subjects not taking oral contraceptives are in agreement with previously reported values of a $t_{1/2}$ of 5.2–6.8 h and oral

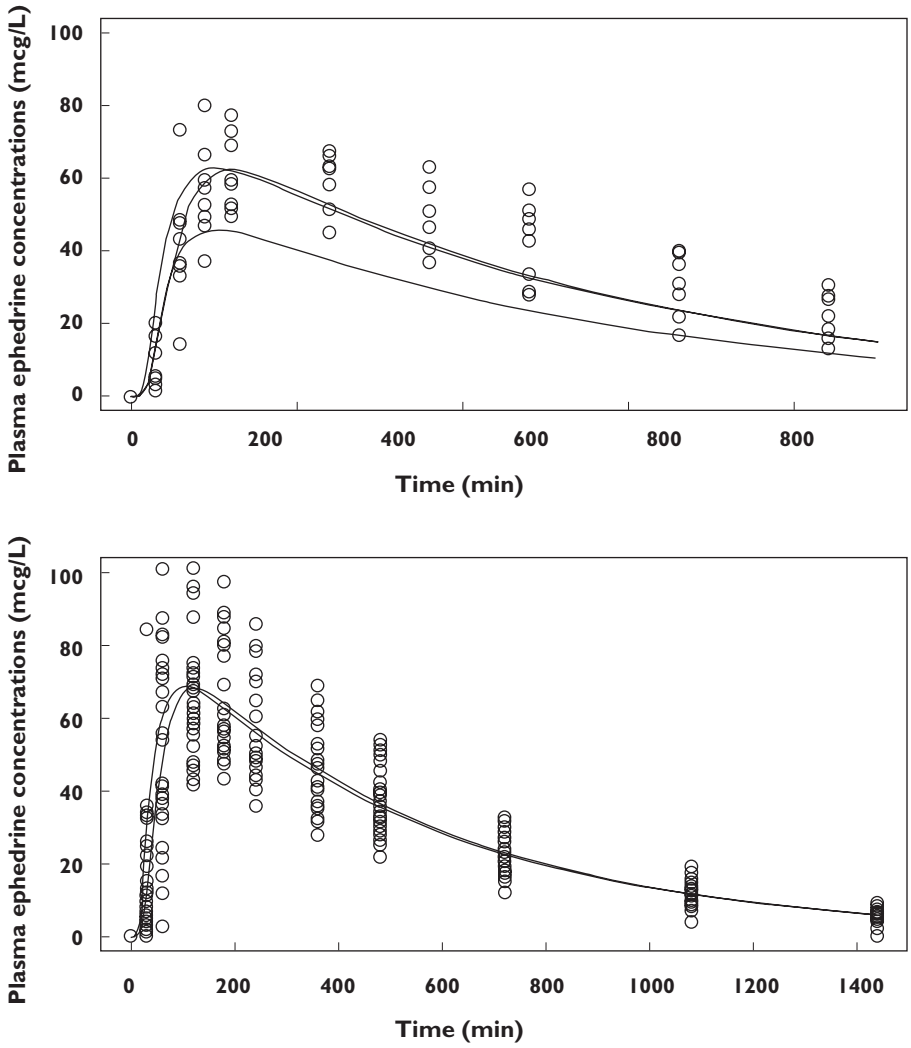

Figure 4
Plasma concentrations (open circles) and population prediction (solid line) for ephedrine. Upper panel: after intake of the herbal formulation. lower panel: after intake of the pharmaceutical formulation. Solid lines: predictions obtained by the final model (equation 10b). Dashed lines: predictions obtained without including the effect of caffeine on absorption. Dotted line: predictions assuming the same relative bioavailability for the two formulations

clearance of 3.9–5.6 l h^{-1} [13, 14, 23, 24]. Caffeine metabolism via CYP1A2 is inhibited by a number of drugs, including oral contraceptives [25]. Thus, an expected decrease in caffeine oral clearance of 46% resulting in an increased $t_{1/2}$ of 11.7 h was observed in subjects taking oral contraceptives. This value is in close agreement with the a $t_{1/2}$ for caffeine in oral contraceptive users, previously reported to be 10.7 [26]. No clear differences in the pharmacokinetic profile of caffeine were observed between the two formulations, except for the presence of a lag-time after administration of the herbal form, which may be related to the presence of interacting compounds in this formulation. No significant influence of ephedrine on the disposition of caffeine was observed.

Ephedrine is mostly excreted unchanged in the urine, but is also metabolized to norephedrine to a small and variable extent, involving enzymatic N-demethylation and oxidative deaminiation of the side chain [27, 28]. Our study is the first to characterize the pharmacokinetics of formation of norephedrine in humans and to demonstrate that a saturable process might be involved in the metabolism of ephedrine to norephedrine. The variance in Km was relatively large, indicating substantial variation in the extent of metabolism of ephedrine to norephedrine. This pathway, however, is minor compared with the renal elimination of ephedrine and, even after repeated drug intake, significant drug accumulation would not be expected.

Renal, renal insufficiency or overdose could lead to the accumulation of ephedrine to potentially toxic effects. Our estimate of CL_R of 0.34 l min^{-1} is consistent with our previously reported data of 0.39 l min^{-1} [14] but this is higher than the value of 0.24 l min^{-1} estimated after intake of herbal ephedrine [13]. It is possible that the other constituents of the herb formuu-

Table 2
Summary of the model selection steps characterizing ephedrine, norephedrine and caffeine pharmacokinetics.

	OF	ΔOF	Action
Caffeine			
Baseline model*	5324		
Including (+)lag time	5307	−17	accepted
+ effect of formulation on lag-time	5288	−19	accepted
+ effect of formulation on bioavailability	−4	rejected	
+ effect of oral contraceptives on clearance	5275	−13	final
Ephedrine, Norephedrine			
Baseline model†	2374		
+ lag time	2296	−78	accepted
+ effect of formulation on bioavailability	2270	−26	accepted
+ Michaelis-Menten metabolization to norephedrine	2146	−124	accepted
+ effect of caffeine on the absorption of ephedrine:			
as dichotomus variable (equation 10a)	2022	−124	accepted
as a function of caffeine in the plasma compartment (equation 10c)	+44	rejected	
as a function of caffeine in the absorption and central compartments (equation 10d)	+44	rejected	
as a function of caffeine in the absorption compartment (equation 10b, 10e)	1963	−59	Final

**1 compartment model with 1st order absorption; †1 compartment model with 1st order absorption and linear metabolization to norephedrine and first order elimination to urine.OF, objective function value; ΔOF, change in the objective function relative to a previously accepted model; Action indicates when a model is accepted or rejected based on the decrease in OF. To facilitate the visual identification of the models that were accepted during the model selection procedure the OF of rejected models is not shown.*

lation may be ressponsible for this decreased clearance. The $t_{1/2}$ of 6.1 h is in close agreement with other reported values, namely 4.8–6.5 h [29], 5.7–6.8 h [30] and 6.1 h [15] after administration of several different herbal and pharmaceutical formulations of ephedrine. Although the half-life of herbal ephedrine appears to be similar to that in its pharmaceutical counterpart, we observed differences in relative bioavailability between the two formulations. The mechanism of this effect is not clear but might indicate that other constituents of the herb formulation have increased the amount of ephedrine absorbed.

We observed an inverse correlation between urine pH and the renal clearance of ephedrine. This suggests that at high urine pH, ephedrine is un-ionized and is easily reabsorbed from the renal tubules, whereas at low urine pH, ephedrine is charged and is thus cleared faster.

Because ephedrine is a weak base (pKa 9.6), it is almost exclusively ionized at low gastric pH and absorption probably occurs in the more alkaline environment of the small intestine. In contrast, caffeine absorption is not pH-dependent (pKa 0.8) and is rapidly diffused through gastric membranes [31].

Although the effects of caffeine on gastric emptying have been controversial [32], a recent study of a herbal caffeine preparation showed a significant prolongation of gastric emptying time compared with placebo [33]. Thus, the decrease in the rate of ephedrine absorption in the presence of caffeine might be explained by a delayed gastric emptying. This hypothesis is supported by the finding that the decrease in the absorption profile of ephedrine was best predicted by the concentrations of caffeine in its absorption compartment and not in the central compartment. Moreover, because the absorption half-life of ephedrine is short, the influence of caffeine would be expected to produce its maximal effect during the early phase of caffeine absorption, when the concentrations are highest. However, the effect of concomitant caffeine on the concentration-time profile of pharmaceutical and herbal ephedrine was small and probably of little clinical relevance.

In conclusion, we have described a novel mechanistic model for ephedrine, norephedrine, and caffeine pharmacokinetics. The effect of caffeine on the pharmacokinetic profile of ephedrine was small and probably of little clinical relevance. It is of interest that

Table 3
Population parameter estimates for ephedrine, norephedrine and caffeine

		Ephedrine		
	Population mean		Intersubject variability*	
Parameter	Estimate	SE† (%)	Estimate (%)	SE‡
V/F (Ll)	181	8	19	43
ka (l min^{-1})	0.036	22	63	57
CLRE/FE(l min^{-1})	0.34	5	11	75
θe	19.5	39		
δ	0.99	–		
kaE,50 (μg)	31.4	57		
FE,pharm	0.59	9		
FE,herbal	0.78	7		
Lag time (min)	16.7	22	47	100
σ (CV%)††	17	50‡	15	46‡
		Caffeine		
	Population mean		Intersubject variability*	
Parameter	Estimate (%)	SE† (%)	Estimate	SE‡ (%)
CLC/FC (l min^{-1})	0.083	9	38	52
θ_{OC}^{CL} d	0.54	16		
V/F (l)	38.6	5	20	55
ka (l min^{-1})	0.064	14	50	66
Lag time (min)	22.2	8		
		Norephedrine		
	Population mean		Intersubject variability*	
Parameter	Estimate	SE† (%)	Estimate	SE‡ (%)
V_{max}/V_{NE} (μg min^{-1} l^{-1})	1.96^{-4}	12	11	75
Km (μg)	2.77	45	43	73
ke_{NE} (l h^{-1})	0.037	45	45	56
σ (CV%)††	15	37‡		

*E, ephedrine; NE, norephedrine, C, caffeine; CL_o/F_o, apparent clearance; V/F, apparent volume of distribution; ka, absorption rate constant; ke, elimination rate constant; t, such that $\delta = exp^{\theta}/(1 + exp^{\theta})$ is the fractional decrease in ephedrine absorption in presence of caffeine $ka_{E,50}$, amount of caffeine in the absorption compartment obtaining δ/2; $F_{E,pharm}$, $F_{E,herbal}$, apparent bioavailabilities for the pharmaceutical and herbal formulations of ephedrine; V_{max}/V_{NE}, apparent maximal rate of metabolism; Km, Michaelis-Menten constant. *Estimates of variability expressed as a coefficient of variation (CV%). †Standard error of the estimates, expressed as coefficient of variation (CV%). ‡Standard error of the variance components, taken as $\sqrt{(SE_{estimate} / estimate)}$, expressed as a percentage. §Where $CL_C(1-\theta_{OC}^{CL}OC)$, and OC = 1 if concurrent treatment with oral contraceptives and 0 otherwise. **Where δ in equation (10b) depends on θ, that is $\delta = {}^{exp\theta}/(1 + {}^{exp\theta})$. ††Residual intrasubject variability of the plasma concentrations, expressed as coefficient of variation (CV%).*

the ingestion of *Ma Huang* extract led to higher concentrations of ephedrine compared with the pharmaceutical forumlation, apparently due to differences in bioavailability. Although we observed saturable metabolism of ephedrine to norephedrine, this represents a minor path of ephedrine elimination. Renal insufficiency or repeated high doses of *Ma Huang* could however, lead to accumulation of ephedrine to potentially toxic concentrations.

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